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Review

# Human adult cardiac autonomic innervation: Controversies in anatomical knowledge and relevance for cardiac neuromodulation☆

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ABSTRACT

*Background:* Cardiac sympathetic blockade is a therapeutic approach for arrhythmias and heart failure and may be a beneficial effect of high thoracic epidural anesthesia. These treatments require detailed knowledge of the spatial location and distribution of cardiac autonomic nerves, however, there are controversies on this subject in humans.

*Objective:* To provide a systematic overview of current knowledge on human anatomy of the cardiac autonomic nervous system.

*Results:* In contrast to the often claimed assumption that human preganglionic sympathetic cardiac neurons originate mainly from thoracic spinal segments T1–T4 or T5, there is ample evidence indicating involvement of cervical spinal segment C8 and thoracic spinal segments below T5. Whether cervical ganglia besides the stellate ganglion play a role in transmission of cardiac sympathetic signals is unclear. Similarly, there is debate on the origin of cardiac nerves from different thoracic ganglia. Most human studies report thoracic cardiac nerves emerging from the first to fourth thoracic paravertebral ganglia; others report contributions from the fifth, sixth and even the seventh thoracic ganglia. There is no agreement on the precise composition of nerve plexuses at the cardiac level. After years of debate, it is generally accepted that the vagal nerve contributes to ventricular innervation. Vagal distribution appears higher in atria, whereas adrenergic fibers exceed the number of vagal fibers in the ventricles.

*Conclusion:* Anatomy of the human cardiac autonomic nervous system is highly variable and likely extends beyond generally assumed boundaries. This information is relevant for thoracic epidural anesthesia and procedures targeting neuronal modulation of cardiac sympathetic innervation.

## 1. Introduction

A balanced function of the cardiac autonomic nervous system (cANS) is essential to maintain cardiovascular homeostasis. The sympathetic cANS has been attributed an important role in the perioperative stress response induced by surgery and anesthesia but is also implicated in the genesis and maintenance of atrial and ventricular arrhythmias (Schwartz et al., 1992; Shen and Zipes, 2014; Looi et al., 2015) and in heart failure (Shuang et al., 2008; Florea and Cohn, 2014). Thoracic epidural anesthesia (TEA) and paravertebral blockade are regularly employed as analgesic techniques. Besides sensory and motor blockade, they also induce blockade of sympathetic cardiac innervation (Fig. 1). This reduces the cardiac chronotropic and inotropic state, the occurrence and magnitude of which seem to vary between individuals and conditions (Wink et al., 2014). In heart failure, an increased sympathetic tone is considered to underlie a chain of effects with detrimental impact on prognosis (Florea and Cohn, 2014; Cohn et al., 1984; Anand et al., 2003). Several arrhythmias are related to an imbalance of autonomic innervation. Blockade of cardiac sympathetic innervation improves myocardial blood flow and oxygen balance during stress

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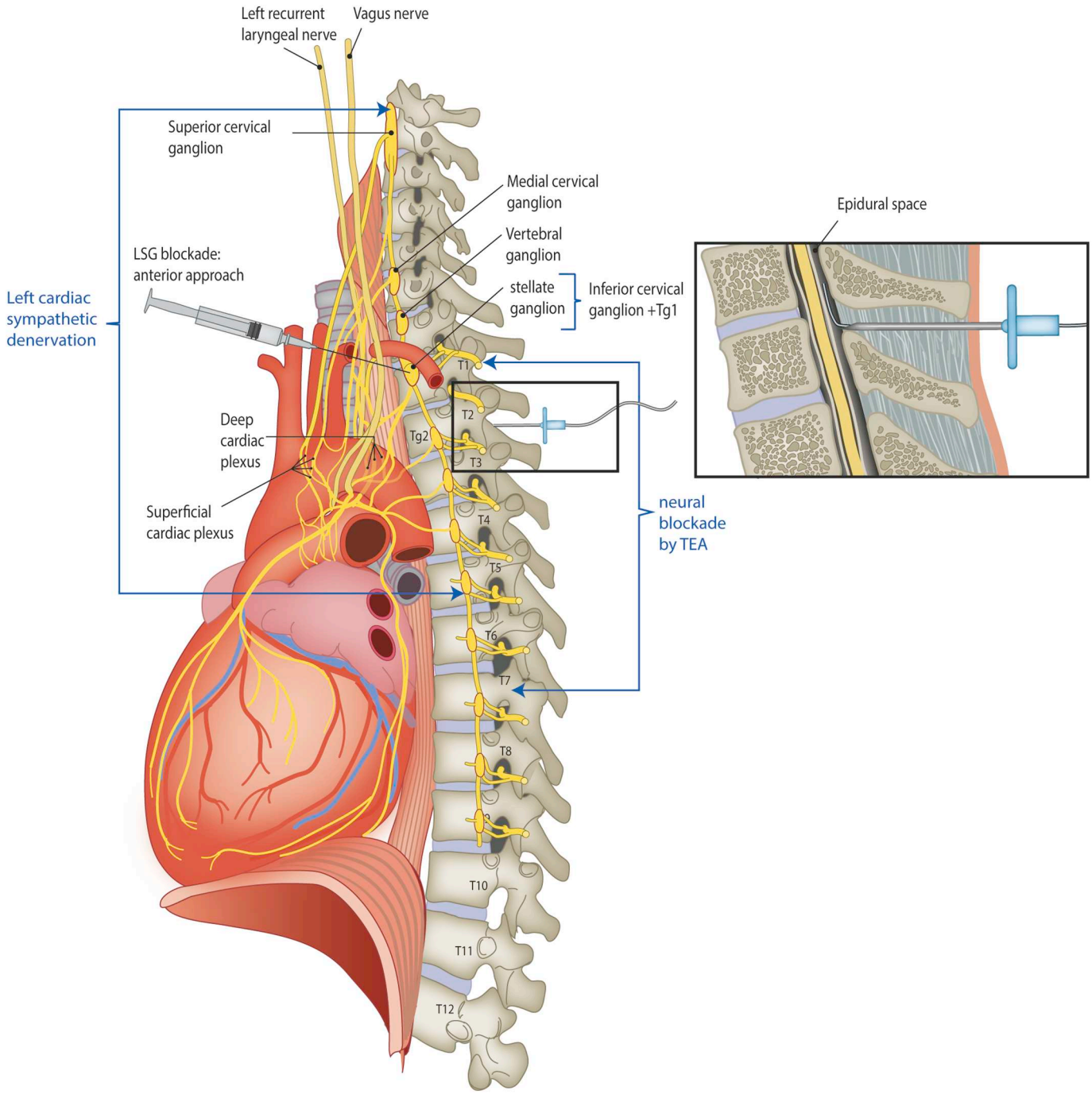



**Fig. 1.** Relationship between level of neuronal modulation and sympathetic input to the heart.
Preganglionic cardiac sympathetic axons synapse with postganglionic sympathetic neurons in the cervical or upper thoracic ganglia (Tg); postganglionic fibers from these ganglia form the sympathetic cardiac nerves that innervate the heart via the deep and superficial cardiac plexus. Neuronal modulation of cardiac sympathetic innervation is achieved by TEA at the spinal level, by left stellate ganglion (LSG) blockade, by blockade of the upper thoracic ganglia, or by paravertebral blockade (i.e. blockade of sympathetic chain ganglia). TEA with upper border of analgesia above spinal segment T7, but certainly above T5, includes blockade of the sympathetic cardiac nerves. The amount of spinal levels blocked depends on the dose of local anesthetic drugs administered epidurally.

(Nygard et al., 2005), and is a novel therapeutic approach for arrhythmias and heart failure (Nygard et al., 2005; Ogawa et al., 2007; Olausson et al., 1997; Do et al., 2017; Ma et al., 2017; Ajijola et al., 2012).

These techniques are aimed at targeting cardiac autonomic innervation, however controversies regarding the anatomy of the human cANS still exist. State of the art information on the anatomy of cardiac sympathetic innervation is relevant for understanding cardiovascular effects elicited by cardiac sympathetic denervation. Furthermore, anatomical variability in human cardiac innervation may contribute to inter-individual diversity in physiological effects and cardiovascular side effects of targeted cardiac sympathetic blockade.

This review aims to provide an update on current knowledge on anatomy of the cANS and focusses on controversies as well as gaps in knowledge on this subject with reference to the clinical practice of neuraxial modulation. The cANS may vary by species (Kawashima, 2011; Kawashima and Thorington Jr, 2011). Hence, this review focusses on the *human* anatomical arrangement of sympathetic outflow

from the spinal cord and sympathetic ganglia to the heart, its heterogeneity and inter-individual variability. The cardiac sympathetic innervation in other animals will not be systemically discussed.

## 2. Methods

The databases Pubmed, Embase and Web of Science were searched by the first two authors (J.W., R.vD.) and by an independent expert librarian (J.W.M.P.) to identify studies that focus on postnatal morphology of the *normal human cANS*, from the preganglionic cardiac sympathetic neurons of the intermediolateral cell column of the spinal cord to the heart as end-organ. Regulation by higher neuronal levels was not part of this review. The following search strategy was developed for PubMed and subsequently adapted for use in the other databases: ("innervation" [Subheading] OR innervat*[ti] OR re-innervat*[ti] OR "nerve"[ti] OR "nerves"[ti] OR "nervous"[ti] OR "neural"[ti]) AND ("sympathetic"[tw] OR "sympathic"[tw] OR "autonomic"[tw]) AND ("Heart"[majr] OR "Heart"[ti] OR "cardiac"[ti] OR "epicardial"[ti] OR "epicardiac"[ti] OR "Pericardium"[majr] OR "epicardium"[ti] OR "intracardiac"[ti] OR "extracardiac"[ti]) AND ("anatomy and histology"[Subheading] OR "anatomy"[tw] OR "anatomy"[MeSH] OR "anatomic"[tw] OR "anatomical"[tw]) NOT ("Animals"[Mesh] NOT "Humans"[Mesh]) NOT (Clinical Study [ptyp] OR Clinical Trial [ptyp] OR Controlled Clinical Trial[ptyp] OR "trial"[tw] OR "trials"[tw]) AND (Dutch[lang] OR English[lang] OR German[lang]).

Language restrictions were Dutch, English and German and the species was limited to Humans.

There were no restrictions concerning age, sex or date of publication. This initial search strategy (performed in the first week of November 2019) yielded 2461 references and 7 additional references through cited references.

The primary focus of the current review is on postnatal macroscopic anatomy; however for description of the intrinsic cardiac nervous system immunohistochemical data (not included in Table 1) were used as well. Studies focussing *solely* on physiology, pathology, embryology, microscopy, histology and molecular biological findings were excluded. (Historical) studies that could not be retrieved, letters to the editor, studies without abstracts and review studies were also excluded.

The authors (J.W. and R.vD.) assessed titles and abstracts and selected articles according to relevance and to the inclusion and exclusion criteria. The remaining articles were reviewed full-text and screened for eligibility (Flowchart 1) according to the inclusion and exclusion criteria by three authors (J.W., R.vD. and M.R.M.J.). During analysis of these reports, special emphasis was placed on discrepancies and variations in anatomy. In addition the methodological quality of the included studies was scored independently by two authors (J.W. and M.R.M.J.) using the QUACS scale (Quality Appraisal for Cadaveric Studies) (Wilke et al., 2015).

## 3. Results

### 3.1. Search results

The search results and study selection flowchart is reported in Fig. 1. From the initial 2468 records identified through database searching and cited reference searching, 62 were duplicates and 2374 records were excluded because the studies did not meet our inclusion criteria. After full-text screening of the remaining 32 records, only 13 studies were eligible for inclusion in this review (Flowchart 1, Table 1). The methodological quality of the included studies was first rated independently by two authors (J.W. and M.R.M.J.) using the Quality Appraisal for Cadaveric Studies (QUACS scale). After comparing and discussing the individual QUACS scores for each article, a consensus score was achieved for each of the 13 articles included in this review (Table 2). In none of the articles, study limitations were clearly defined. Study limitations mentioned in Table 2 were derived from discussion sections. We scored education of dissecting researcher positive when authors experience was clear from previous publications in this field referred to in the paper.

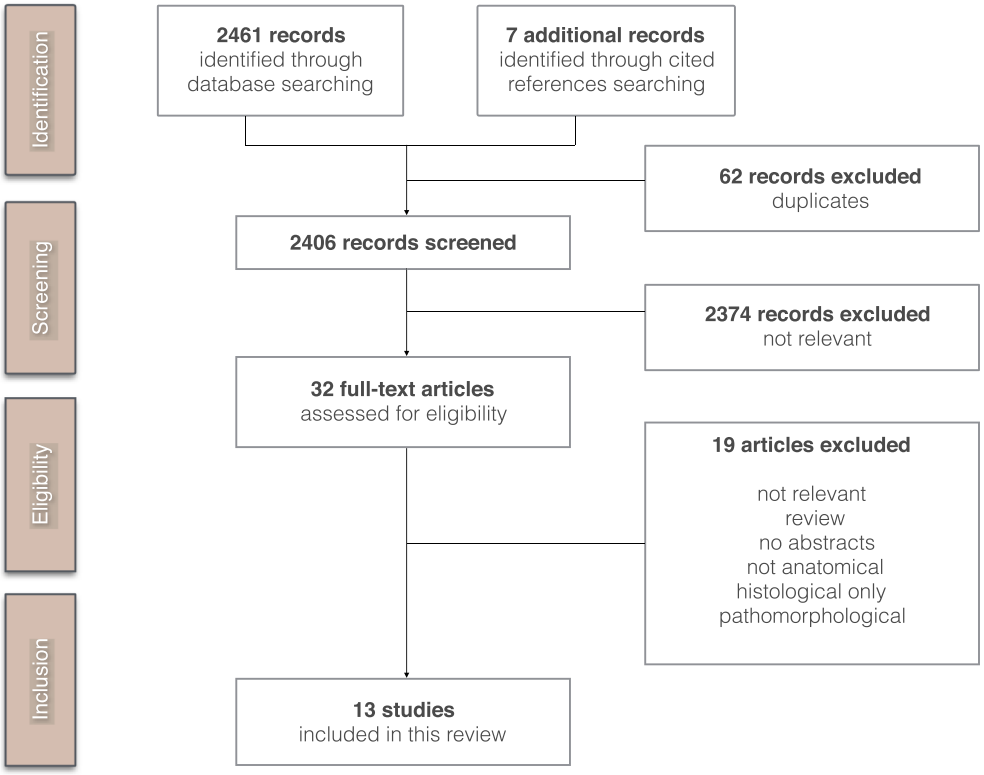
### 3.2. Extrinsic cardiac sympathetic nervous system

#### 3.2.1. Output from the spinal cord towards the sympathetic chain

Only few morphological studies on human output of the spinal cord towards the sympathetic chain were identified. In 1968, Bonica documented that the human preganglionic cardiac sympathetic output from the spinal cord originates from the first to the fourth, and sometimes fifth thoracic spinal cord segment (Bonica, 1968). This study is often cited in TEA studies as an anatomical reference of cardiac sympathetic innervation, and is to our knowledge the only human document to date that describes cardiac sympathetic innervation from the level of the spinal column in humans (Bonica, 1968) It was this study (not listed in Table 1 because it is a review article) that was the initial inspiration to perform this systematic review. Fibers of preganglionic sympathetic neurons in the intermediolateral cell column contributing to cardiac sympathetic autonomic innervation leave the spinal cord in the anterior roots, after which they enter the spinal nerves, pass through the anterior rami and travel via white rami communicantes towards the paravertebral ganglia of the sympathetic chain, where they synapse on postganglionic neurons (Figs. 2, 3). Preganglionic neurons may synapse with many multiple postganglionic neurons (Bonica, 1968).

*3.2.1.1. Controversy regarding levels of sympathetic output from spinal cord to sympathetic ganglia.* Some research suggests that sympathetic nerves may also emerge higher, from the cervical myelum (Sheehan, 1941). Although there is a clear transition from C8 to T1 in the amount of sympathetic fibers, Sheehan in 1941 observed sympathetic fibers in the anterior roots of C8 of humans (Fig. 2). Evidence of sympathetic fibers was based on the identification of small (below 3 μ in diameter) myelinated fibers. However, the amount of cervical roots fibers was scarce, especially compared to the anterior roots of T1-T2 (Sheehan, 1941). Moreover, presence of sympathetic fibers at level C8 does not necessarily imply that these are cardiac destined fibers. Therefore, it remains unclear whether the cervical myelum delivers cardiac destined autonomic fibers and if so, their significance is unclear. Determination of the upper border of spinal autonomic outflow to the heart could possibly be identified by physiological studies. These studies however, would be challenging in humans.

Controversy also exists on the *level of synapse* of preganglionic cardiac sympathetic fibers within the sympathetic chain. The sympathetic preganglionic neurons of spinal cord segments *T1–T5* have been described to either synapse in the first sympathetic ganglion they reach or to ascend within the sympathetic chain to synapse in a ganglion at a higher level, particularly to the 3rd–4th cervical ganglia (Bonica, 1968). However, sympathetic preganglionic neurons of spinal level *T6* to as low as *T10* have been described to either terminate in the first sympathetic ganglion they reach, ascent or even descent to a higher or lower thoracic ganglion (Bonica, 1968) (Fig. 3). This raises the question from which thoracic spinal cord levels the preganglionic sympathetic fibers innervating the heart originate. A limitation is that aforementioned studies are dated several decades ago, when techniques e.g. for neuronal tracing and specific immunohistochemical labelling were limited available, or altogether unavailable. Therefore it seems difficult if not impossible to state with certainty that the small fibers described as representing the sympathetic outflow really are of sympathetic origin. Bonica did not describe the method that was used to detail the preganglionic origin of cardiac sympathetic innervation (Bonica, 1968). Moreover, neural tracing techniques as performed in animals cannot be performed in humans. Interpretation of results of immunohistochemical stainings in many cases relies on (alleged) specificity of immunohistochemical stainings to discriminate the different divisions of




**Flowchart 1.** Flow diagram of literature search.

the autonomic nervous system. If preganglionic sympathetic fibers from spinal level T5–T10 indeed ascent to higher paravertebral ganglia, this would imply that thoracic ganglia Tg1–Tg5 could be innervated from spinal levels below T5. From a theoretical point of view, it is not unlikely that preganglionic sympathetic neurons from spinal levels below T5 might be involved in cardiac sympathetic innervation.

In conclusion, the cranial border of spinal sympathetic outflow to the heart is most likely confined to T1 in most cases. However, sympathetic outflow from the cervical myelum has been described for C8 and to date it remains unclear whether this is a common variation and whether spinal cervical sympathetic outflow is involved in cardiac sympathetic innervation (see question marks in Fig. 2). Therefore the upper border of preganglionic sympathetic neurons originating from the spinal cord to the paravertebral ganglia providing the heart with postganglionic sympathetic fibers, might involve cervical spinal segment C8 as well. Similarly, the lower border of preganglionic cardiac sympathetic neurons may involve spinal levels below T5. This information might be relevant in case where for instance TEA is targeted to block all spinal cardiac sympathetic segments.

The clinical relevance of these considerations is that complete blockade of cardiac sympathetic innervation may require blockade of spinal segments below T5. This is relevant since TEA blocks sympathetic outflow at the spinal level. Another consequence would be that involvement of sympathetic cardiac segments in neural blockade may, besides high- TEA, more likely apply to mid-TEA (often used in abdominal surgical procedure) with cranial spread of anesthetic blockade.

### *3.2.2. Sympathetic ganglia giving rise to cardiac postganglionic output, the sympathetic cardiac nerves*

The sympathetic chain consists of paravertebral ganglia situated bilaterally on the anterolateral side of the vertebral column. Here, preganglionic sympathetic neurons synapse to cell bodies of the postganglionic sympathetic neurons that extent via *cardiac nerves* to the heart (Fig. 2).

The cervical ganglia, receiving preganglionic sympathetic input from the thoracic spinal cord via ascending fibers within the thoracic ganglia, are an extension of the sympathetic chain (Bonica, 1968; Kawashima, 2005; Janes et al., 1986; Kawashima and Sasaki, 2007). Although nomenclature differs in literature over the past 50 years, the most accepted names are: *superior cervical ganglion*, *middle cervical ganglion*, *vertebral ganglion* and *inferior cervical ganglion* (Fig. 2). The inferior cervical ganglion, fused with the first thoracic ganglion in about 80% of humans to form the cervicothoracic or stellate ganglion (Kawashima, 2005; Pather et al., 2006; Zhang et al., 2009), is generally accepted to provide postganglionic cardiac nerves (Fig. 2). A high incidence of double cardiac sympathetic nerves arising from this ganglion has been demonstrated by Pather et al. (2006). They reported a 97.9% incidence of two rami arising from the stellate ganglion, possibly representing the inferior cervical cardiac ramus and the first thoracic cardiac ramus.

Postganglionic nerves to the heart from the cervical and thoracic sympathetic chain do not travel via gray rami communicantes with the spinal nerves but originate as separate cardiac nerves from the paravertebral ganglia (Fig. 2). The cardiac sympathetic nerves enter the heart through the vascular pole. At the arterial pole, cardiac nerves extend along the common carotid, subclavian and brachiocephalic arteries towards the aorta and branches also extend along the pulmonary trunk. At the venous pole, cardiac nerves run along the superior vena cava. Thoracic cardiac nerves descend obliquely along the thoracic vertebrae or the intercostal vessels, sometimes following complex courses through the mediastinum, before reaching the heart (Kawashima, 2005).

*3.2.2.1. Controversy regarding involvement of cervicothoracic ganglia and nerves in cardiac innervation.* As stated earlier, Bonica reported preganglionic cardiac sympathetic outflow to emerge from cervical and the upper four to five thoracic spinal cord segments and postganglionic cardiac sympathetic outflow from the upper five thoracic paravertebral ganglia (Bonica, 1968). However, there is controversy on the exact origin of (postganglionic) sympathetic

**Table 1**
Postnatal human morphological studies on cardiac autonomic innervation.

| Author | Year | Title | Number/sides | Anatomic region | | |
|---|---|---|---|---|---|---|
| | | | | Spinal level – sympathetic trunk | Sympathetic trunk-superior/ deep cardiac plexus | Epicardial ganglionated plexus |
| Sheehan (1941) | 1941 | Spinal autonomic outflows in man and monkey | 9 adults/18 sides | + | − | − |
| Saccomanno (1943) | 1943 | The components of the upper thoracic sympathetic nerves | Not reported | − | + | − |
| Janes et al. (1986) | 1986 | Anatomy of human extrinsic cardiac nerves and ganglia | 23 adults/46 sides | − | + | − |
| Singh et al. (1996) | 1996 | Topography of cardiac ganglia in the adult human heart | 15 adults | − | − | + |
| Armour et al. (1997) | 1997 | Gross and microscopic anatomy of the human intrinsic cardiac nervous system | 18 adults | − | − | + |
| Pauza et al. (2000) | 2000 | Morphology, distribution, and variability of the epicardiac neural ganglionated subplexuses in the human heart | 21 hearts (3 fetuses, 9 neonates/children, 9 adults) | − | − | + |
| Pather et al. (2003) | 2003 | The sympathetic contributions to the cardiac plexus | 8 adults and 21 fetuses/58 sides | − | + | + |
| Kawashima (2005) | 2005 | The autonomic nervous system of the human heart with special reference to its origin, course, and peripheral distribution | 18 adults/36 sides | + | + | + |
| Pather et al. (2006) | 2006 | Cervico-thoracic ganglion: it's clinical implications | 31 fetuses/60 sides<br>17 adults/29 sides | + | + | − |
| Kawashima and Sasaki (2007) | 2007 | Morphological comparison of the cardiac autonomic nervous system between normal and abnormal great arterial branching pattern with a brief review of the literature | 26 adults/52 sides | − | + | − |
| Zhang et al. (2009) | 2009 | Anatomical variations of the upper thoracic sympathetic chain | 25 adults/50 sides | − | + | − |
| Petraitiene et al. (2014) | 2014 | Distribution of adrenergic and cholinergic nerve fibers within intrinsic nerves at the level of the human heart hilum | 9 adults | − | − | + |
| Watanabe et al. (2016) | 2016 | Gross anatomical study on the human myocardial bridges with special reference to the spatial relationship among coronary arteries, cardiac veins, and autonomic nerves | 60 adults | − | − | + |

**Table 2**
Description and assessment of the included studies.

| Study | Objective | Subjects | | | Methods | | Results | Limitations reported | QUACS score (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | n | Age (years) | Sex (M/F) | Type | Condition, solution and staining or histochemistry | Outcome | | |
| Sheehan (1941) | To check extent and variations in spinal autonomic outflow | 9 | 30–80 | 8/1 | Autopsy cadavers<br>Morphologic/ histologic | Health: NR<br>Preservation: 10% formol saline<br>Staining: osmic acid vs 1% silver nitrate solution | Size and number of myelinated fibers in the ventral roots | Final proof of spinal autonomic outflow is from physiological evidence | 75% |
| Saccomanno (1943) | To assess the numbers, sizes, and anatomical relationships of cardiac sympathetic nerves | NR | NR | NR | Autopsy and dissecting room cadavers<br>Morphologic/ Histologic | Health: NR<br>Preservation: osmic acid<br>Staining: Bodian's technic (silver impregnation) | General morphologic description of sympathetic cardiac nerves | NR | 50% |
| Janes et al. (1986) | To describe anatomy of the extrinsic cardiac nerves and ganglia | 23 | 6–89 | 15/8 | Autopsy (n = 10) and embalmed (n = 13) cadavers<br>Morphologic/ histologic | Health: NR<br>Preservation: Embalming fluid; 25% glycerine, methylhydrate, 6% phenol, 6% formaldehyde and water<br>Fixation: ethanol-benzene-paraffin<br>Staining: Cresyl violet or Cresyl violet with Luxol vast blue | General morphologic description and microscopic anatomy of cervical and thoracic autonomic nerves and ganglia | NR | 67% |
| Singh et al. (1996) | To determine the distribution of intrinsic cardiac ganglia | 15 | 35–66 | 2/3 | Heart tissue from autopsy cadavers and heart transplant procedures<br>Morphologic/ histologic | Health: only normal hearts (complete)<br>Preservation: 10% formalin<br>Fixation: ethanol-xylene-paraffin<br>Staining: Harris' hematoxylin and eosin Y | Topography and ordinal ranking of cardiac ganglia density in different heart regions | NR | 92% |
| Armour et al. (1997) | To determine the distribution of intrinsic cardiac ganglia and their connectivity | 18 | 28–69 | NR | Autopsy cadavers<br>Morphologic/ histologic | Health: NR<br>6 complete hearts<br>Preservation: 10% formalin<br>Staining: 1% methylene blue, 5% uranyl acetate, toluidine blue and lead citrate | The location and number of major intrinsic cardiac ganglia including neural connectivities and their estimated neuronal component | It was not possible to visualize all the neurons in larger ganglia | 83% |
| Pauza et al. (2000) | To determine the distribution of intrinsic cardiac ganglia and their connectivity | 9<br>12 | 22–74<br>< 6 | 8/13 | Autopsy cadavers<br>Morphologic/ histologic | Health: specimens without cardiac complications<br>Complete hearts<br>Preservation: 4% paraformaldehyde solution<br>Staining: Karnovsky-Roots medium | The location and number of major intrinsic cardiac ganglia including neural connectivities and their neuronal component | Fat pads in the epicardium makes counting of ganglia more difficult | 85% |
| Pather et al. (2003) | To determine the cervical and thoracic sympathetic contributions to the cardiac plexus | 8<br>21 | 18–55<br>< 0 | 4/4<br>11/10 | Type cadavers NR<br>Morphologic/ Histologic | Health: NR<br>Preservation: formalized (Solution NR)<br>Staining: hematoxylin and eosin<br>Fixation: paraffin | Incidence and origin of cervical and thoracic sympathetic cardiac rami | NR | 58% |
| Kawashima (2005) | To clarify the detailed morphology of the entire autonomic cardiac nerves | 18 | Adults (age NR) | NR | Embalmed cadavers<br>Morphologic/ histologic | Health: hearts/vessels with abnormal anatomy excluded<br>Preservation: 10% formalin and 30% ethyl alcohol<br>Staining: Sudan black solution | Incidence, origin of all cardiac nerves and their peripheral distribution | NR | 67% |
| Pather et al. (2006) | Incidence and distribution of the cardiothoracic ganglion | 48 | 21 fetus<br>17 adults<br>37–64 | NR | Type cadavers NR<br>Morphologic/ histologic | Health: NR<br>Preservation: NR<br>Staining: hematoxylin and eosin | Incidence, location and morphology of the cardiothoracic ganglion | NR | 46% |
| Kawashima and Sasaki (2007) | To compare anatomy of the cardiac autonomic nervous system between humans with normal and with anomalous arterial branches | 27 | NR | NR | Embalmed cadavers<br>Morphologic/ histologic | Health: anatomically normal hearts and normal arterial branches (n = 26)<br>Anatomically normal hearts and anomalous arterial branches (n = 1)<br>Preservation: 10% Formalin and 30% ethyl alcohol<br>Staining: no staining | General morphology and incidence and origin of the cardiac autonomic nervous system | The right thoracic cardiac nerves were difficult to trace | 75% |

(*continued on next page*)

**Table 2** (*continued*)

| Study | Objective | Subjects | | | Methods | | Results | Limitations reported | QUACS score (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | n | Age (years) | Sex (M/F) | Type | Condition, solution and staining or histochemistry | Outcome | | |
| Zhang et al. (2009) | To delineate the upper thoracic sympathetic chains and their neural connections with ventral rami of the thoracic nerves | 25 | NR | 18/7 | Embalmed cadavers<br>Morphologic | Health: NR<br>Preservation: embalmed and fixed with formaldehyde solution (Solution NR)<br>Staining: no staining | Morphology of the upper sympathetic trunk and its neural connections to the intercostal nerves or thoracic nerves | NR | 62% |
| Petraitiene et al. (2014) | To evaluate the quantitative distribution of TH- and ChAT-positive cardiac axons within intrinsic nerves at the level of the heart hilum | 9 | 22–76 | 5/4 | Autopsy cadavers<br>Morphologic/ histologic | Health: hearts without cardiac pathology<br>Preservation: 4% formaldehyde solution (pH 7.4) in 0.01 M PBS, consisting of (mM): $Na_2HPO_4$, 8.06; $NaH_2PO_4$, 1.94; KCl, 2.7 and NaCl, 137 in high-purity distillate $H_2O$.<br>Staining: immunohistochemistry: for AChE, TH and CHAT | The nerve areas and densities of TH-immunoreactive and ChAT-immunoreactive fibers and relative proportions of adrenergic and cholinergic fibers were compared between subplexuses | NR | 77% |
| Watanabe et al. (2016) | To elucidate the spatial relationship among the coronary arteries, cardiac veins and autonomic nerves at myocardial bridges | 60 | 59–101 | 36/24 | Autopsy cadavers<br>Morphologic/ histologic | Health: macroscopically abnormal hearts were excluded<br>Preservation: 10% formalin and 40% ethyl alcohol<br>Histochemistry: for TH and CHAT | Incidence and distribution of myocardial bridges and the spatial distribution of coronary arteries, veins and autonomic nerves within these bridges | NR | 75% |

QUACS, Quality Appraisal for Cadaveric Studies.
ChAT, choline-acetyltransferase; TH, tyrosine hydroxylase; NR, not reported.

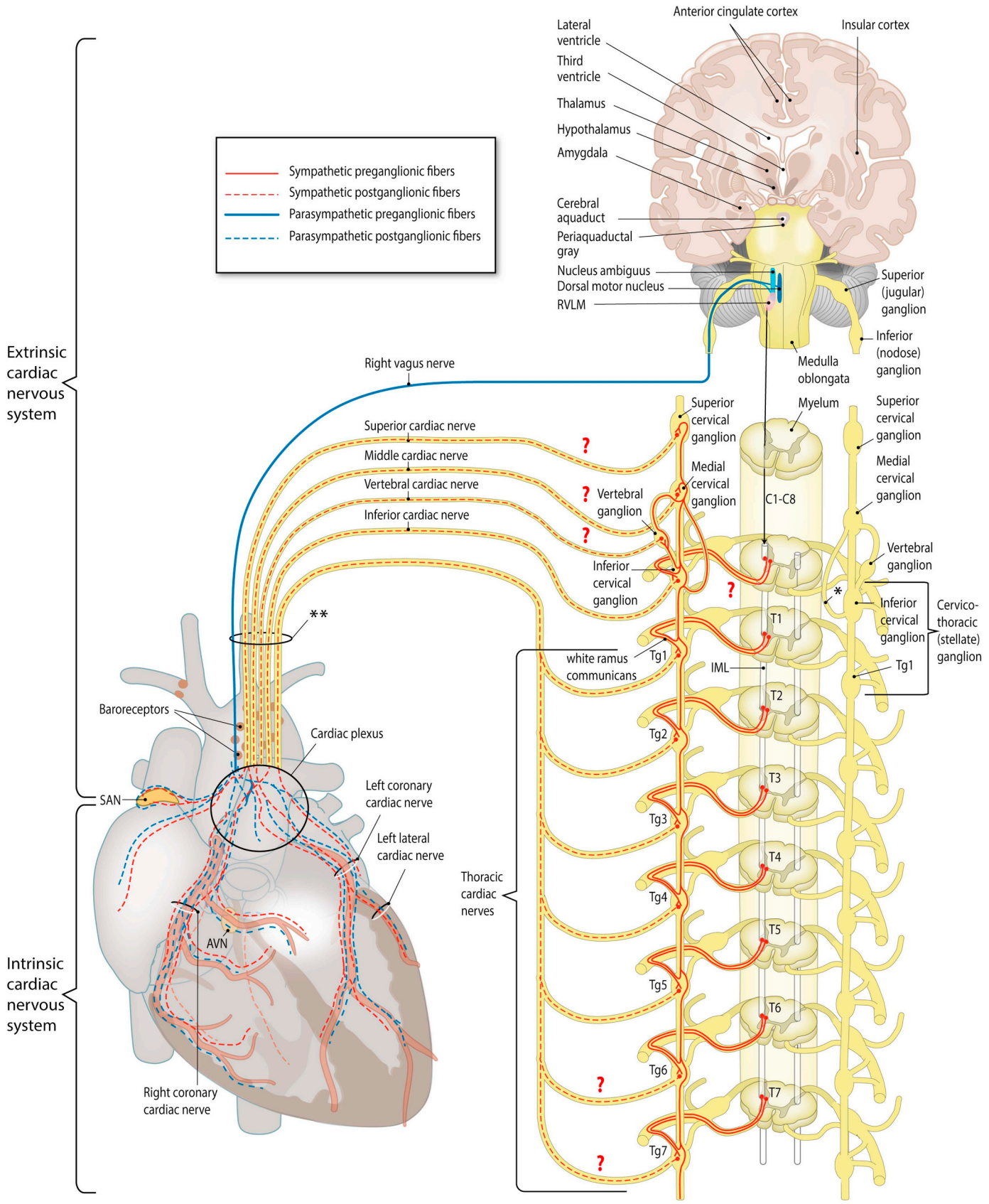
cardiac innervation. Anatomical studies on human cadavers show variability and inconsistency in their reported upper and lower limits of origin of postganglionic cardiac sympathetic innervation. Where many authors report that thoracic cardiac nerves emerge from the first to fourth thoracic ganglia (summarized in Kawashima, 2005), others reported contributions from the fifth thoracic (Bonica, 1968), sixth thoracic (Pather et al., 2003) and even from the seventh thoracic ganglia (Saccomanno, 1943) to the thoracic cardiac nerves (Fig. 2).

Despite extensive anatomical research, the route and even number of postganglionic nerves remains unclear. Kawashima described an important connection between the superior cervical ganglion and the heart, by the so called *superior cardiac nerve* (Kawashima, 2005; Kawashima and Sasaki, 2007). He further described the *middle cardiac nerve* originating from the middle cervical and the vertebral ganglion, and the *inferior cardiac nerve* originating from the inferior cervical/cervicothoracic (stellate) ganglion. Besides these cervical ganglia, he reported that each of the upper 4–5 thoracic paravertebral ganglia has a sympathetic connection towards the heart, sharing the combined name '*thoracic cardiac nerves*' (Fig. 2) (Kawashima, 2005). This study confirms the previously reported cervical and thoracic sympathetic contributions to the cardiac plexuses by Saccomanno (1943) and Pather et al. (2003). Anatomically, the contributions of thoracic cardiac nerves to cardiac innervation may be quite important since these nerves were shown to comprise twice as many nerve fibers as the cervical sympathetic cardiac nerves (Saccomanno, 1943). By contrast, Janes et al. reported no cardiopulmonary nerves arising from the superior cervical ganglia and sympathetic chain inferior of the stellate ganglion (Janes et al., 1986). He states that cardiopulmonary nerves only arise from the stellate ganglia and the caudal halves of the cervical sympathetic chains (Janes et al., 1986), leaving no role for transmission of sympathetic input to the heart for the superior cervical ganglion nor the thoracic paravertebral ganglia (Fig. 2). These authors also report the cardiopulmonary nerves to contain mediastinal ganglia along their course. The nature of these ganglionic neurons i.e. being postganglionic sympathetic or local circuit neurons is unknown.

In conclusion the origin of cardiac sympathetic innervation, i.e. the level of the sympathetic ganglia giving rise to postganglionic cardiac nerves, has been shown to differ between anatomical studies and inter-individual variations may occur. Methodological differences and lack of human physiological studies partly explain the contrasting results. Whether cervical ganglia besides the stellate ganglion play a role in transmission of cardiac sympathetic signals is unclear. Similarly, there is debate on the origin of cardiac nerves from different thoracic ganglia. In some patients, thoracic ganglia *Tg6* and *Tg7* (see question marks in Fig. 2) might be involved in cardiac sympathetic innervation. If so, preganglionic sympathetic neurons from spinal segments T6 and T7 or, if ascending, even from spinal segments below T7 are likely to be involved in cardiac sympathetic innervation. In addition, these anatomical studies demonstrated inter-individual and intra-individual variety (asymmetry in left to right sympathetic innervation) in the anatomy of cardiac autonomic innervation. These variations may be relevant in procedures targeting the cardiac output to the heart, e.g. during left cardiac sympathetic denervation targeting the lower cervical/upper thoracic nerves.

### 3.2.3. Combination of cardiac nerves to form mixed cardiac plexuses

Postganglionic sympathetic and preganglionic parasympathetic (branches of vagus and recurrent laryngeal) nerves converge at the cardiac surface to form plexuses (Kawashima, 2005; Kawashima and Sasaki, 2007) (Fig. 1). The *superficial (ventral) cardiac plexus* is located near the aortic arch and the left pulmonary artery on the left side and near the ascending aorta and brachiocephalic trunk on the right side. The *deep (dorsal) cardiac plexus* is located between the aortic arch and the tracheal bifurcation (Pather et al., 2003). It is questionable whether the distinction between the deep and superficial plexus truly can be made (Pather et al., 2003).




*(caption on next page)*

**Fig. 2.** Overview of cardiac innervation.
Schematic drawing of the cardiac autonomic nervous system. Preganglionic cardiac parasympathetic axons arise from neurons in either the nucleus ambiguus or dorsal vagal nucleus; they run in cardiac branches of the vagus nerve (blue, solid lines) to synapse in cardiac plexuses and ganglia from where postganglionic fibers (blue, dotted lines) innervate the sino-atrial node (SAN), atrioventricular node (AVN), coronary arteries and ventricular myocytes. Preganglionic cardiac sympathetic axons (red, solid lines) arise from neurons in the intermediolateral cell columns (IMLs) of the upper four or five (possibly six or seven) thoracic spinal segments, that receive modulating input from several forebrain centers (e.g. the insular cortex, anterior cingulate cortex, central nuclei of the amygdala, and several hypothalamic nuclei interneurons); they leave the spinal cord through anterior (ventral) roots, enter the anterior (ventral) rami of spinal nerves and pass to the sympathetic chains through white rami communicantes to synapse in the upper thoracic (Tg) or cervical ganglia; postganglionic fibers (red, dotted lines) from these ganglia form the sympathetic cardiac nerves. At the heart parasympathetic and sympathetic nerves converge to form the cardiac plexus from which atrial and ventricular autonomic innervation is arranged.
The red question marks (?) indicate anatomical structures of which existence and/or involvement in cardiac sympathetic innervation are debated.
*Ansa subclavia.
**Vagal and sympathetic nerves intermingle before reaching the cardiac plexus and projecting on the heart.

### 3.3. Intrinsic cardiac nervous system

Upon entering the pericardial sac, mixed autonomic nerves project to cardiac ganglia that are interconnected by local circuit neurons, thus forming *ganglionated or epicardial neural plexuses* at the cardiac arterial and venous poles. These plexuses are embedded in the epicardial fat (Fig. 4). The (postganglionic) sympathetic fibers either directly innervate the myocardium or first synapse on the intrinsic cardiac ganglia located in the myocardial wall and epicardial fat. The (preganglionic) parasympathetic fibers all synapse on intrinsic cardiac ganglia (Kapa et al., 2010). Ganglia consist of a heterogeneous population of neurons of afferent, efferent and local circuit neurons (Palma and Benarroch, 2014; Yuan et al., 1993; Armour and Hopkins, 1990; Blomquist et al., 1987). The largest number of ganglia is located at multiple sides near the atria. Ventricular ganglia are mostly distributed in the epicardial fat near the aortic root and adjacent to major branches of the coronary arteries (Armour et al., 1997; Pauza et al., 2000; Singh et al., 1996). With a total amount of cardiac ganglia between 706 and 1506 and an estimated amount of neurons in the epicardial neural plexus between 14,000 and 43,000, the human intrinsic cardiac nervous system is very extensive (Armour et al., 1997; Pauza et al., 2000). The highly interconnected and integrated cardiac ganglia have intrinsic activity that is modulated by sympathetic or parasympathetic (vagal) inputs (Palma and Benarroch, 2014). Therefore cardiac ganglia are not simply relay stations but rather local intergrational centers where input is modulated. These plexuses thus contain mixed cardiac nerves, i.e. nerves originating from different cardiac sympathetic nerves but also from parasympathetic nerves. The use of markers such as tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) has helped elucidate the composition of adrenergic and cholinergic intrinsic cardiac neurons and nerves in the heart. Next to morphological data (Table 1), immunohistochemical data were used for description of the intrinsic

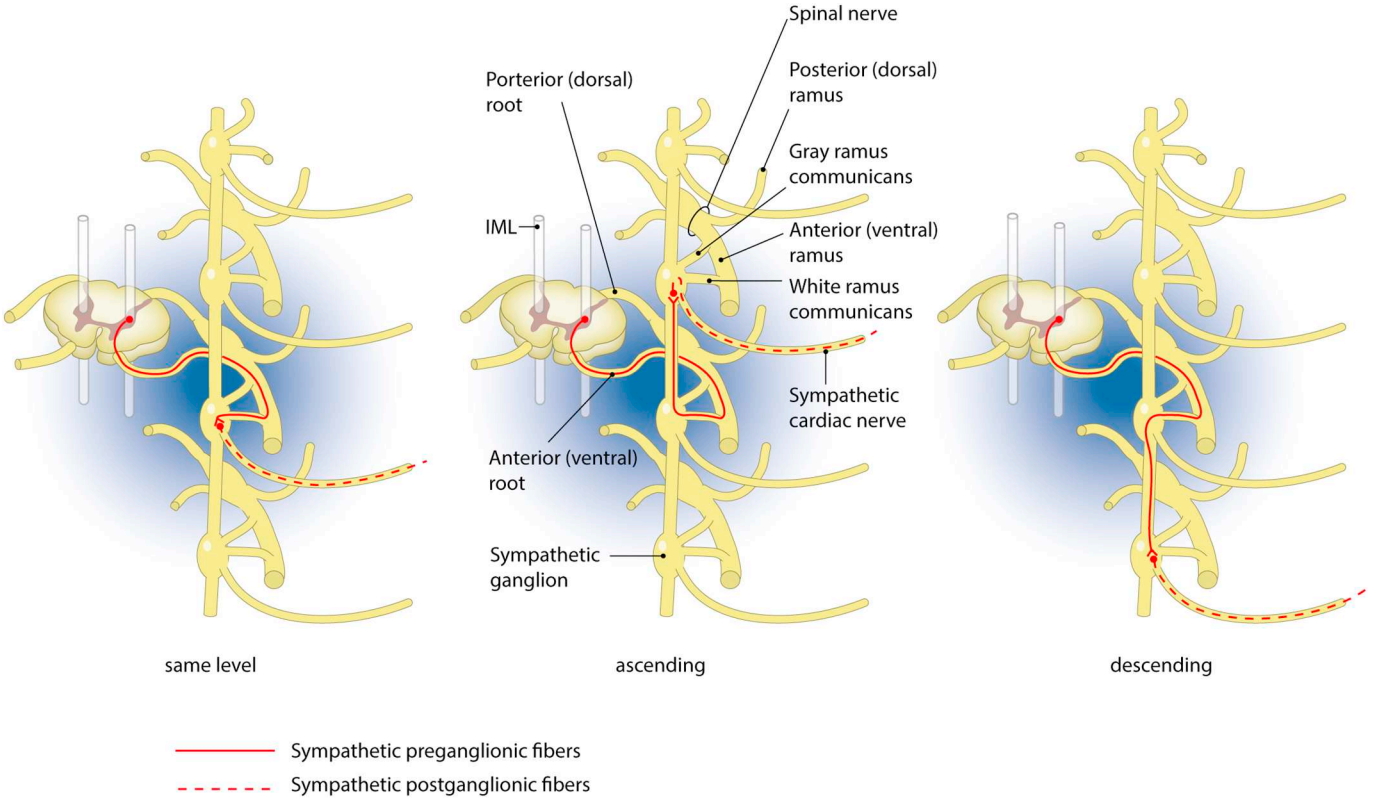



**Fig. 3.** Courses taken by preganglionic sympathetic fibers.
After leaving the spinal cord via the anterior (ventral) root, the axons of preganglionic sympathetic neurons enter the anterior (ventral) ramus of the spinal nerve and pass to the sympathetic chain through a white ramus communicans. Within the sympathetic chain preganglionic fibers may 1) synapse immediately with a postganglionic neuron of the paravertebral ganglion at that level, 2) ascend in the sympathetic chain to synapse with a postganglionic neuron of a higher paravertebral ganglion, or 3) descend to synapse with a postganglionic neuron of a lower paravertebral ganglion.
IML, intermediolateral cell column of the spinal cord.

cardiac nervous system.

Petraitiene et al. (2014) obtained tissue samples of intrinsic nerves from seven ganglionated plexuses from human hearts as described by Pauza et al. (2000): the left and right coronary subplexuses, the ventral right atrial and ventral left atrial subplexuses, the left dorsal subplexus, the middle dorsal subplexus, the dorsal right atrial subplexus. They demonstrated that autonomic fibers to the ganglionated plexuses innervating the right atrium predominantly contain cholinergic fibers. In contrast, plexuses innervating the left atrium and left and right ventricle are predominantly innervated by adrenergic fibers (Petraitiene et al., 2014). Besides innervation from cholinergic and noradrenergic nerves, intrinsic ganglia receive innervation by a host of neurochemically distinct nerves (Hoover et al., 2009). Neurons of human atrial ganglia receive input from nerves displaying immunoreactivity for neuronal nitric oxide synthase (nNOS), substance P (SP), calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP) (Hoover et al., 2009). These findings support the concept of the intrinsic cardiac ganglia as complex neural networks with highly regulated control of regional cardiac function.

Three main large (mixed sympathetic and parasympathetic) nerves that follow the coronary arteries or their major branches (Watanabe et al., 2016) can be recognized that contribute to innervation of the atria and ventricles: the *left coronary cardiac nerve* (runs along the anterior interventricular branch of the left coronary artery), the *left lateral cardiac nerve* (runs along the circumflex artery) and the *right coronary cardiac nerve* (runs along the right coronary artery) (Janes et al., 1986). Additional cardiopulmonary nerves connect to these (coronary) cardiac nerves distal from the plexuses, innervating coronary vessels and myocardial cells. Both cholinergic and adrenergic nerves run from the epicardium into the myocardium (Kawano et al., 2003). However, there are more cholinergic nerves at the subendocardial than at the subepicardial area of the myocardium (Saburkina et al., 2009). In accordance with Petraitiene et al. (2014), Kawano et al. (2003) report that atria are more densely innervated by cholinergic nerves whereas the ventricles are predominantly innervated by adrenergic fibers.

### 3.3.1. *Innervation of the cardiac conduction system*

The atrioventricular node and sinoatrial node are more densely innervated than the His bundle and bundle branches, although the latter cardiac conduction system components still receive more innervation than the adjacent ventricular myocardium (Crick et al., 1994). There is an initial sympathetic dominance in nerve supply of the conduction system in childhood, with gradual transition into a sympathetic and parasympathetic co-dominance in adulthood (Kawano et al., 2003; Chow et al., 2001; Chow et al., 1995).

### 3.3.2. *Controversies in distribution of cholinergic and adrenergic nerve fibers and location of ganglionated plexuses*

The cardiac topography of the intracardiac ganglionated plexuses, consisting of numerous ganglia on atria and ventricles, seems to be according to a pattern. The epicardial neural plexus has been described as a system of six to 10 subplexuses localized at discrete cardiac regions (Armour et al., 1997; Pauza et al., 2000) (Fig. 4). Armour et al. identified five atrial and five ventricular locations containing ganglionated plexuses (Armour et al., 1997). The group of Pauza et al. described a system of seven subplexuses observed at five atrial and two ventricular locations (Pauza et al., 2000). The ganglionated plexuses are interconnected suggesting that a plexus might have interaction with several topographic regions of the heart (Armour et al., 1997). Although ganglionated plexuses were observed to be located at specific cardiac regions, variability seems to exist in the location of ganglia.

The results of the studies of Kawano et al. (2003) and Petraitiene et al. (2014) are partly conflicting since Petraitiene reported that the left atrium is predominantly innervated by adrenergic nerve fibers where according to Kawano it is more densely innervated by cholinergic fibers.

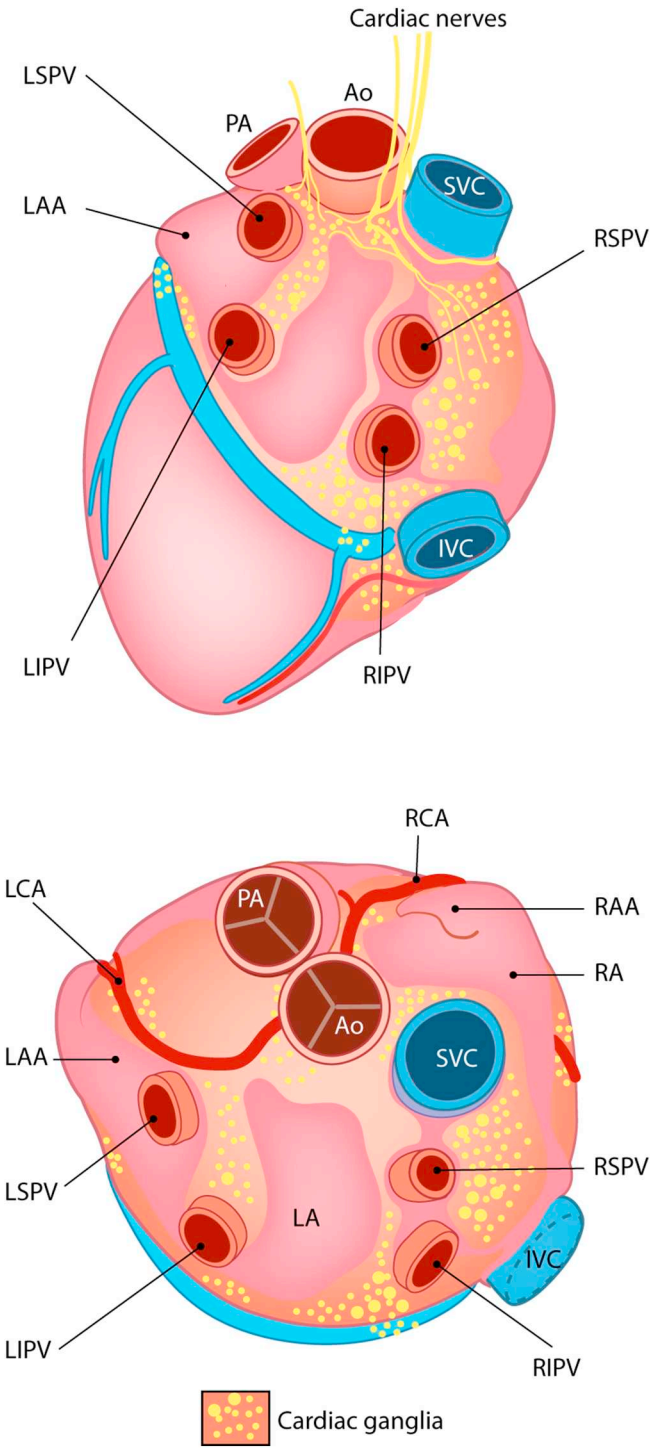



**Fig. 4.** Cardiac plexuses.
Drawing of a postero-inferior and a superior view of the human heart illustrating the distribution of ganglionated plexuses on the surface of the atria and ventricles. Modified after Armour et al. (1997).
Ao, aorta; IVC, inferior vena cava; LA, left atrium; LAA, left atrial appendage; LCA, left coronary artery; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; Pa, pulmonary artery; RA, right atrium; RAA, right atrial appendage; RCA, right coronary artery; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; SVC, superior vena cava.

With regard to innervation of the myocardium, there is controversy on the density of autonomic innervation in atrial versus ventricular tissues. Parasympathetic (vagal) nerves appear to be more densely

distributed in the atria at both neonatal and adult stages (Kawano et al., 2003; Chow et al., 1995). After years of debate, it is now generally accepted that the vagus nerve also contributes to ventricular innervation (Coote, 2013). Several human immunohistochemical studies concluded that cholinergic (vagal) nerves not only innervate the human atria and cardiac conduction tissues but also the ventricles (Pauza et al., 2000; Kawano et al., 2003; Kent et al., 1974).

Some authors describe equal densities of sympathetic nerve fibers in atria and ventricles, at least in the neonatal stage (Chow et al., 1995), whereas other authors describe more sympathetic nerves in the ventricles in the adult heart (Kawano et al., 2003). This might indicate that differentiation of the cANS still occurs after birth, supported by observation of dynamic changes in nerve supply throughout life (Chow et al., 2001).

## 4. Clinical correlations

### 4.1. Physiology and anatomy of cardiac innervation

Anatomically it seems that the spinal cord at T1–T3, which incorporates 1/3 of approximately 90,000 preganglionic sympathetic neurons of the thoracic myelum (Coote, 1988) (not specifically cardiac sympathetic neurons), would be an important contributor of sympathetic outflow to the heart. Interestingly, the thoracic cardiac nerves reported to deliver the most substantial contribution to the cardiac plexus are the third and fourth (Ellison and Williams, 1969) or the fourth and fifth thoracic segments (Saccomanno, 1943). However, the amount of neurons does not necessarily correlate to the strength of the physiological effects elicited after stimulation of these nerves. In addition, sympathetic nerve fibers have also been demonstrated in human vagal nerves (Seki et al., 2014).

Physiological studies examining the effects of electrical stimulation of sympathetic nerves from different spinal cord levels can provide information on the contribution of different spinal segments to cardiac innervation. However, these studies are lacking in humans. There are human studies describing electrophysiologic and hemodynamic responses to stimulation of the cervico-thoracic ganglia. Randall and McNally assessed elevations in blood pressure and cardiac acceleration after stimulation of separate ganglia of the upper thoracic sympathetic chain during surgery (Randall and McNally, 1960). Elevations in heart rate and blood pressure were reported after stimulation of thoracic ganglia Tg1–Tg5, but not after stimulation below Tg5 (cervical levels were not included in this study). Considerable variation was found in thoracic levels of cardiac sympathetic innervation in different patients. Similarly, there was variability in which thoracic ganglion elicited the strongest response. In humans, stimulation of the left stellate ganglion (LSG) has been shown to elicit increases of contractile forces in the left ventricle and increases in blood pressure (Ajijola et al., 2015; Zhou et al., 2013). In addition, LSG stimulation results in shortening of the RVOT activation recovery interval and QT interval and increases spatial dispersion of repolarization, which is assumed to be associated with increased risk of ventricular arrhythmias (Ajijola et al., 2015). In humans with myocardial infarction, heterogeneity in repolarization was greater after sympathetic stimulation compared to normal hearts (Vaseghi et al., 2012a). By contrast, vagal nerve stimulation has been shown to reduce ventricular contractility and ventricular arrhythmia inducibility (Vaseghi et al., 2017; Lewis et al., 2001).

Besides variation in which ganglion evokes the strongest cardiac response, differential effects from left and right sided cardiac sympathetic structures have been described. LSG and right stellate ganglion (RSG) stimulation in humans have differential effects on heart rate reflected by a more substantial increase in heart rate after RSG stimulation compared to LSG stimulation (Ajijola et al., 2015; Rogers et al., 1978).

### 4.2. Correlation with animal studies

Several animal studies using transneural retrograde labelling support the observation of Bonica (Bonica, 1968) that preganglionic sympathetic neurons can ascent or descent to a higher or lower ganglion from where postganglionic fibers are projected towards the heart. Markers injected in the stellate ganglion of rat were found in spinal segments *C8* to *T8* with a peak at T2 (Pyner and Coote, 1994). Whether these spinal segments are involved in *cardiac* sympathetic innervation or that other regions are targeted remains to be elucidated. The latter study also suggests that the axons of sympathetic preganglionic neurons from one spinal cord segment may branch and have axonal projections to multiple cervical and thoracic ganglia. In another study, rat hearts were injected with retrograde tracers, which were subsequently found in the preganglionic sympathetic neurons of spinal cord levels *T1–T7* and in some rats even in spinal levels *T8–T11* (Ter Horst et al., 1996).

Electrophysiological studies in animals reveal that each cervical or thoracic paravertebral ganglion is innervated by a subset of spinal segments. Ganglia are usually most strongly innervated by one particular spinal segment with contributions from adjacent spinal segments that diminish as a function of distance from the dominant segment (Szulczyk and Szulczyk, 1987; Lichtman et al., 1980). In cats, maximal evoked responses in several cardiac nerves were demonstrated after stimulation of T2 and responses gradually decreased after stimulation of T1, T3, T4 and T5 (Szulczyk and Szulczyk, 1987). Another study in guinea pigs demonstrated that the majority of superior cervical ganglion cells receive input from spinal segments T1–T4, the majority of stellate ganglion cells from spinal segments T2–T6 and the majority of fifth thoracic ganglion cells from T4-T8 with a main supply from spinal level T5. Although the majority of segments involved in innervation of the fifth thoracic ganglion arose from the T4–T8 spinal segments, occasionally even spinal segments T9 and T10 were involved (Lichtman et al., 1980).

A study in pigs revealed that the both the LSG and RSG provide significant innervation of the anterior wall of the left ventricle (Vaseghi et al., 2012b). Both LSG and RSG stimulation increased activation recovery interval (ARI), a surrogate measure of action potential duration. Also dispersion in ARI is increased by both LSG and RSG stimulation, providing mechanistic evidence for beneficial effects of left and potentially right sympathectomy in reducing arrhythmias (Vaseghi et al., 2012b; Vaseghi et al., 2013).

Animal studies also support the idea that cardiac ganglia are not simply relay stations but rather local intergrational centers where input is modulated. A study in anesthetized mongrel dogs demonstrated spontaneous activity in neurons located in the ganglionated plexus in epicardial fat which was related to the cardiac and respiratory cycle. This spontaneous activity was influenced by sympathetic and parasympathetic innervation as well as by input from cardiac mechanoreceptors (Armour and Hopkins, 1990). Another study in anesthetized mongrel dogs displays that multiple sites within the intrinsic cardiac nervous are involved in sinoatrial nodal regulation (Randall et al., 2003). Consequently, blockade or ablation potentially disrupts these processes.

### 4.3. Modulation of the cardiac sympathetic nervous system as a treatment intervention in cardiac arrhythmias and heart failure

Heart failure and cardiac arrhythmias are associated with increased sympathetic tone and central inhibition of sympathetic tone might be beneficial. Risk factors for ventricular arrhythmias are prolongation of the QTc interval as well as prolongation of transmural dispersion of repolarization (TDR). Owczuk and colleagues demonstrated that thoracic but not lumbar epidural anesthesia results in significant decreases of the QTc interval and TDR (Owczuk et al., 2009). Electrocardiographic changes following blockade of the human RSG or LSG comprised prolongation of the QT interval. However, blockade of the RSG

appeared to exert asymmetric or opposite effects to those of the LSG, suggesting a predominance of the left over the right SG (Cinca et al., 1985). These results suggest that TEA or blockade of LSG/RSG might have antiarrhythmic potential. Indeed, TEA and stellectomy significantly reduced arrhythmia burden in patients with refractory ventricular arrhythmias in structural heart disease (Ajijola et al., 2012; Bourke et al., 2010). Further support for a protective role of TEA is provided by a case-report demonstrating TEA as successful treatment in a patient with electrical storm not responding to conventional treatment (Mahajan et al., 2005).

Cardiac sympathetic denervation may also be a valuable adjunctive treatment for atrial fibrillation (AF). After blockade of the LSG or RSG, atrial effective refractory period prolonged, AF was less inducible and episodes were of shorter duration. In patients who underwent sympathetic ganglion blockade, the effect of right and left blockade was equivalent and the impact on each atrium was comparable regardless of the site of block (Leftheriotis et al., 2016). In addition, catheter-ablation of AF likely also affects the cardiac ganglia, which may improve the procedural success rate, at least at short term (Scherschel et al., 2019).

In patients with severe chronic heart failure due to dilated cardiomyopathy, the use of high TEA as an adjunct to medical treatment was associated with reduced chamber dimensions, improved systolic cardiac function and reversed myocardial fibrosis (Ma et al., 2017; Guo et al., 2012).

Depending on the technique of neuromodulation, cardiac sympathectomy might also result in off-target effects. In contrast to other forms of cardiac sympathetic blockade, the application of TEA as a therapeutic option in cardiac disease, enables to block *all* sympathetic input to the heart. However, if epidural blockade extends beyond T1-T5, this will result in venous and arterial dilatation with reduction in preload and afterload and consequently hypotension (Veering and Cousins, 2000).

Beyond the scope of this review, but of interest, is the potential therapeutic management of heart disease by targeting non-cholinergic or non-noradrenergic neuropeptides like neuronal nitric oxide synthase (nNOS), substance P (SP), calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP) (Hoover et al., 2009).

## 5. Conclusions and clinical implications

The exact origin of preganglionic sympathetic neurons innervating the human heart is controversial and remains a matter of debate. Although human cardiac sympathetic innervation is regularly described to emerge from spinal cord segments T1–T4 or T5, several human anatomical studies report involvement of lower levels. Sympathetic outflow from the cervical myelum has been described for C8 although to date it remains unclear whether spinal cervical sympathetic outflow is involved in cardiac sympathetic innervation.

Along with the stellate ganglion, other cervical paravertebral ganglia may be involved in the transduction of cardiac sympathetic signals, and there is still debate on the origin of cardiac nerves from different thoracic ganglia. In some patients, thoracic ganglia *Tg6* and *Tg7* might be involved in cardiac sympathetic innervation. If so, preganglionic sympathetic neurons from spinal segments T6 and T7 (or even from spinal segments below T7) are likely to be involved in cardiac sympathetic innervation. Therefore, complete blockade of cardiac sympathetic innervation may require blockade of spinal segments below T5 or thoracic ganglia below Tg5 and cervical ganglia besides the stellate ganglion. Another consequence would be that involvement of sympathetic cardiac segments in neural blockade may, besides high-TEA, more likely apply to mid-TEA (often used in abdominal surgical procedure) with cranial spread of anesthetic blockade. Besides ambiguous cranial and caudal borders of cardiac sympathetic innervation there is considerable inter-individual and intra-individual (left-right differences) anatomical variation. This variability renders the outcome of procedures targeting neuronal modulation of cardiac sympathetic innervation, such as stellate ganglion and paravertebral blockade, unpredictable.

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## Declaration of competing interest

No conflicts of interest.

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